REMARKS

The September 23, 2004 Official Action and references cited therein have been carefully considered. In view of the amendments submitted herewith and the following remarks, favorable reconsideration and allowance of this application are respectfully requested.

Status of the prosecution:

As a preliminary matter, Applicants request that the Attorney Docket Number of record in this case be changed from "13216-73220" to –RUCC-0046 (98-0087)--.

The oath or declaration has been deemed defective due to the presence of non-initialed alterations. A new oath or declaration has been required. Applicants are in the process of locating the inventors so that a new declaration may be executed. However, inasmuch as four years has now elapsed since the original declaration was executed, this process has not yet been completed. As soon as the inventors are located and can be contacted, a new declaration or oath will be executed and submitted to the Patent Office.

Claims 1-3 and 5-10 are pending and were examined. Claims 1-3 and 5-10 were rejected under 35 U.S.C. §112, second paragraph, for alleged indefiniteness with respect to (1) lack of antecedent basis for certain terms in claims 1 and 3, and (2) alleged omission of essential elements in claim 1.

Claims 1-3 and 5-10 were rejected under 35 U.S.C. §112, first paragraph, for alleged lack of written description for the new term "culturing organogenic."

Claims 1-3 and 5-10 were rejected under 35 U.S.C. §112, first paragraph, for alleged lack of enablement with respect the conditions of inoculating, culturing and selectively culturing in claim 1.

Claims 1-3 and 5-10 were rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Stalker (U.S. 4,810,648) in view of Lee et al. (U.S. 5,948,957.

In accordance with the present amendment, claim 1 has been amended to clarify antecedent basis for "turfgrass plant," and to further clarify the steps of the transformation protocol recited therein. Claim 3 has been canceled.

Applicants respectfully assert that the presently amended claims are in condition for allowance, for the reasons set forth below.

The claims meet the requirements of 35 U.S.C. §112, second paragraph:

Claims 1-3 and 5-10 were rejected under 35 U.S.C. §112, second paragraph, for indefiniteness. The rejection alleged indefiniteness with respect to (1) alleged lack of antecedent basis for "the turfgrass plant" in claim 1 and (2) alleged lack of antecedent basis for "hybrid vector" and "pSB11" in claim 3. Though Applicants disagree with the examiner's allegation that "hybrid vector" and "pSB11" lack antecedent basis, claim 3 has been canceled, rendering the rejection moot. Claim 1 has been amended to "a" turfgrass plant, rather than "the" turfgrass plant. Accordingly, the definiteness requirement in this regard should be deemed fully satisfied.

The rejection also alleged omission of essential elements in claim 1, namely, inoculation conditions and culturing conditions. Claim 1 has been amended to further clarify that the transformation protocol recited therein is a standard *Agrobacterium* co-cultivation protocol, well known to those of skill in the art.[1] Applicants assert that all essential steps of this established protocol are recited in the claims, and the means for carrying out each step would be familiar to the skilled artisan. Accordingly, one of skill in the art would recognize the metes and bounds of the claim. Nothing further is required under the provisions of 35 U.S.C. §112, second paragraph.

For each of the reasons set forth above, Applicants request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, second paragraph.

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1 The co-cultivation technique for *Agrobacterium*-mediated transformation has been practiced for many years. Indeed, it is sufficiently commonplace to be taught in textbooks and laboratory manuals for students. An example of the manner in which such protocols are presented in laboratory manuals may be found in Chapter 22 of Plant Biotechnology, A Practical Approach, by H.S. Chawla, Science Publishers, Inc., Enfield N.H., 2003, a copy of which is submitted herewith.

The subject matter of the claims is enabled by the specification:

Claims 1-3 and 5-10 stand rejected on the ground that the specification does not enable practice of the invention in the scope encompassed by the claims. Specifically, the examiner asserts that, though the specification may recite specifics of the methods of the invention, those limitations are not set forth in the claims, namely, specific conditions associated with the inoculation and culture steps recited in claim 1. The examiner therefore continues to allege that the claims are too broad to practice without undue experimentation. Applicants traverse this rejection as applied to the presently amended claims.

As Applicants have stated previously, the specification teaches certain factors believed important to the success of transforming turfgrass. These include (1) use of strong monocotyledonous promoters within the transformation vector, (2) use of strong *vir* genes, such as those found in the "superbinary" vector systems described by Komari et al. (Plant J. 10: 165-174, 1996) or in U.S. Patent 5,731,179; and (3) use of starting material that produces friable, regenerable callus. Three working examples, representing the claimed method applied to three different turfgrasses, are provided. Claim 1 recites each of those three factors. The remainder of the methodology used to practice the invention as claimed is the common methodology of *Agrobacterium* co-culture, which has been known in the art for many years. *See, e.g.,* Chapter 22 of Plant Biotechnology, A Practical Approach, by H.S. Chawla, Science Publishers, Inc., Enfield N.H., 2003 (hereinafter "the Chawla Lab Manual"), a copy of which is submitted herewith. For illustrative purposes, the table below sets forth the steps of the method taught and exemplified in the present application, as compared with the scope of the currently amended claims and the state of the art as reflected by the Chawla Lab Manual and other scholarly publications.

TABLE: Comparison of method as disclosed and claimed in the present application with "standard" methods as exemplified in Chapter 22 of the Chawla Lab Manual

Step taught in specification	Exemplified?	Recited in claims?	Known in art?
Starting tissue (e.g., seeds) are surface sterilized and placed on standard de-differentiation culture medium for 3-6 weeks, preferably in the dark	YES: Creeping bentgrass (CB), tall fescue (TF), velvet bentgrass (VB): mature seeds placed on MMSG de-differentiation medium, in the dark at room temperature (RT) for 3-6 weeks	1. (a) culturing organogenic tissue from a turfgrass plant on a medium that promotes de-differentiation of the tissue, **to produce regenerable callus tissue**	YES: leaf disc method exemplified in Chawla Lab Manual, and transformation of callus following dedifferentiation of various tissues was known[2]
Proliferating calli (only callus that is friable and regenerable is selected) is transferred to fresh medium of the same type	YES: friable, regenerable callus from CB, TF and VB cultured seeds was selected, and transferred to fresh medium one or more times to promote active growth	See step of claim 1(a) above	This feature (selection of friable, regenerable callus) is believed to contribute to the novelty and non-obviousness of the claimed invention; transfer of callus to fresh medium is a standard technique
The *Agrobacterium* strain carrying the transforming plasmid is grown, induced with acetosyringone (induces *vir* gene expression) and resuspended in an inoculation medium containing acetosyringone	YES: for transformation of CB, TF or VB, *Agrobacterium* strain carrying specified *vir* genes and reporter gene controlled by monocot promoter (Example 1) was used. *Agrobacterium* was grown in AB medium, induced with acetosyringone and kept at RT for 3.5 hours in the dark before use in co-cultivation	Claim 1(b) . . . . inoculating with *Agrobacterium* carrying at least one vector for transformation, **the vector comprising virulence genes from plasmid pSB1 or pSB4**, in which vector is inserted a heterologous DNA construct and a selectable marker conferring antibiotic resistance to transformed cells, wherein the DNA construct and selectable marker are operably linked to a **promoter from a monocotyledonous species** . . . [CONDITIONS FOR GROWING BACTERIA NOT RECITED]	The selection of the recited ***vir* genes** and the **monocot promoter** are believed to contribute to the novelty and non-obviousness of the claimed invention; but the method steps (how to grow *Agrobacterium* and induce *vir* gene expression) were known in the art, as can be seen from pages 190-192 of Chawla Lab Manual.
Callus tissue is placed in presence of *Agrobacterium* to allow the bacteria to	YES: for CB, TF, VB, callus was mixed with pre-induced	1 (b) . . . wherein the inoculating comprises mixing the callus tissue with the	YES: co-culture method was well known, as

[2] *See, e.g.,* Chapter 10 of Plant Biotechnology, A Practical Approach, by H.S. Chawla, Science Publishers, Inc., Enfield N.H., 2003; Komari (1990) Plant Cell Reports 9:303-306; Cheng et al. (1997) Plant Physiol. 115:971-980; copies of each of which are enclosed herewith.

infiltrate the callus. Co-culture proceeds for about 3 days; excess *Agro* can be removed by vacuum filtration	*Agrobacterium* and incubated at RT in the dark for 1.5 hours. For CB and TF, excess bacteria were then removed by filtration. For VB, excess *Agrobacterium* was not removed. Filters with inoculated callus were moved to plates containing MSSG or glucose, plus acetosyringone, and stored in the dark at RT for 3 days.	*Agrobacterium* under conditions permitting the *Agrobacterium* to infiltrate the callus tissue . . .; (c) co-culturing the *Agrobacterium*-infiltrated culturing the inoculated callus tissue under conditions that enable the *Agrobacterium* vector to transform cells of the *Agrobacterium*-infiltrated callus tissue [SPECIFIC CONDITIONS NOT RECITED]	exemplified by Chapter 22 of Chawla Lab Manual and as cited in many articles (for example as cited in Footnote 2). Chawla Lab Manual instructs a protocol similar to the protocol taught in the present application.
Calli transferred to a selection medium containing the selection antibiotic, e.g., hygromycin, and are kept in that medium several (6-8) weeks and checked periodically for proliferation of calli	YES: for CB, TF and VB, co-cultivated calli were rinsed with 250ug/ml cefotaxime to suppress bacterial growth, then placed on agar plates containing MSSG medium, 200 ug/ml hygromycin and 250 ug/ml cefotaxime. Calli were kept 6-8 weeks in the dark at RT and checked periodically for proliferation	1(d): selecting transformed cells by culturing the *Agrobacterium*-infiltrated inoculated callus tissue on a selection medium comprising an antibiotic, wherein the transformed cells are resistant to the antibiotic and are selected by growth in the presence of the antibiotic; [SPECIFIC CONDITIONS NOT RECITED]	YES: as exemplified in Chawla Lab Manual, selection and regeneration method steps were well known, and depend upon the type of tissue that was transformed. Further, the references cited in Footnote 2 set forth similar selection and regeneration schemes.
New growth appearing on calli in the selection medium is transferred to a fresh selection medium and allowed to proliferate	YES: , not specifically stated for CB, but for TF and VB, specification states that calli transferred to MSSG for further proliferation	1(e): regenerating a transformed turfgrass plant from the transformed cells [SPECIFIC CONDITIONS NOT RECITED]	YES: see comment above
After sufficient proliferation, a small portion of the suspected transformed calli is tested for the presence of the transgene, preferably by expression of the gene	YES: for TF and VB, specification states that putatively transformed calli were tested for GUS activity. Zilinskas declaration states that all three varieties were tested for GUS activity and found to contain it	No	YES: as exemplified by the Chawla Lab Manual (pp 192-193, transformation may be confirmed by PCR, Southern hybridization or transient GUS expression assays
Transformed calli are transferred to a regeneration medium containing growth regulators to promote shoot differentiation	YES: for CB, TF and VB, calli transferred to Regeneration Medium I (MSO I) containing hygromycin and cefotaxime, kept in dark for one week, then moved to light for shoot development for about two weeks	See claim 1(e); [SPECIFIC CONDITIONS NOT RECITED]	YES: see comment above

Shoots are transferred to a second regeneration medium to promote root growth, and plantlets thereafter transferred to new medium or to soil	YES: for CB, TF and VB, calli with shoots were transferred to second regeneration medium (MSO II) to promote root development, thereafter transferred to new medium and CB were transferred into soil	See claim 1(e); [SPECIFIC CONDITIONS NOT RECITED]	YES: see comment above

As can be seen from the table, each of the three factors that Applicants assert contribute to the novelty and/or non-obviousness of the claimed invention is recited in the claims, and the steps recited in the claimed method cover the method in its entirety. But, as the table illustrates, specifics of culture conditions for bacteria or plant material throughout the method are *not* recited in the claims because (1) they are taught and exemplified in the specification, and (2) they are standard conditions well known to the skilled artisan at the time this application was filed – so well known, in fact, that they are taught in a laboratory textbook such as the Chawla Lab Manual, as well as in many scholarly articles published before the filing date of the present application (two representative examples of which are referred to in the table).

The examiner has stated that "essential steps and conditions not known to one of ordinary skill in the art are unpredictable, and must be recited in the claims." Applicants respectfully submit that the claims indeed do recite all essential steps and conditions, and that the particulars of any steps and conditions that are not recited were well known in the art, as discussed above. Not everything necessary to practice an invention need be disclosed, much less claimed. The Federal Circuit has stated that what is well known is best omitted. *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991). Further, the scope of enablement must only bear a reasonable connection to the scope of the claims. *See, e.g.*, *In re Fisher*, 427 F.2d 833, 839 (CCPA 1970). In the instant case, in view of the information well known to the skilled artisan, the scope of enablement provided by the specification more than fully bears a reasonable connection to the scope of the currently pending claims. Nothing more should be required under 35 U.S.C. §112, first paragraph.

For at least the foregoing reasons, Applicants assert that the method recited in amended claim 1 is fully enabled by present specification, in view of its teachings and working examples, and the information commonly available to the skilled artisan.

Reconsideration and withdrawal of the rejection of claims 1-3 and 5-10 under 35 U.S.C. 112, first paragraph, is therefore respectfully requested.

The claimed subject matter is not obvious in view of the cited references:

Claims 1-3 and 5-10 stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Stalker (U.S. 4,810,648) in view of Lee et al. (U.S. 5,948,957. According to the examiner, Stalker teaches production of transgenic turfgrass by providing regenerable callus tissue, inoculating the tissue with *Agrobacterium* having a heterologous DNA construct linked to promoter from a monocot species and an antibiotic resistance selectable marker, culturing under conditions enabling the *Agrobacterium* to transform cells of the tissue, *Agrobacterium* virulence genes, selectively culturing on antibiotic, and producing transgenic turfgrass. The examiner acknowledges that Stalker does not teach virulence genes from pSB1 or pSB4, the maize ubiquitin promoters, rice actin promoters, maize Adh1 promoters, rice or maize tubulin promoters and/or alfalfa histone 3 promoters, or the genes encoding glucose oxidase, citrate synthase, a gene encoding a delta-9 desaturase, a gene encoding a delta-11 desaturase, a plant homolog of neutrophil NADH oxidase, a gene encoding bacteriopsin from *H. halobium* or a gene encoding a pokeweed antiviral protein. However, the examiner alleges that Lee et al. teach the use of a rice actin promoter in the production of transgenic turfgrass, utilizing a method involving the transformation of nodal tissue of a stem and cultivation on an antibiotic selection medium. The examiner further alleges that the virulence genes and heterologous genes recited in the claims of the present invention were admittedly known in the art; therefore, the combination of the teachings of Stalker, Lee et al. and the state of the art render the claimed invention obvious. Applicants respectfully, but strenuously, traverse this rejection.

When applying 35 U.S.C. § 103, the following tenets of patent law apply: (1) the claimed invention must be considered as a whole; (2) the reference must be considered as a whole and must suggest the desirability and thus the obviousness of making the modification or combination; (3) the reference must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention; and (4) reasonable expectation of success is the standard with which obviousness is determined. *Hodosh v. Block Drug Co., Inc.*, 786 F.2d 1136, 1143 n.5, 229 USPQ 182, 187 n.5 (Fed. Cir. 1986); MPEP §2141.

These requirements are not met by the cited references, alone or in combination. The claimed invention calls for a method of *Agrobacterium*-mediated transformation of turfgrass, comprising the steps of (1) de-differentiating organogenic tissue to form callus, (2) selecting friable, regenerable callus, (3) co-culturing the selected callus with *Agrobacterium* harboring a transformation vector comprising virulence genes from plasmid pSB1 or pSB4, in which is inserted a heterologous DNA construct and a selectable marker conferring antibiotic resistance to transformed cells, wherein the DNA construct and selectable marker are operably linked to a promoter from a monocotyledonous species, (4) selecting callus containing transformed cells on a selection medium, and (5) regenerating plants from the transformed cells. In order to establish a *prima facie* case of obviousness, each of these limitations must be taught or suggested by the cited references. They are not.

The examiner has acknowledged that Stalker does not teach *Agrobacterium* vectors having *vir* genes from pSB1 or pSB4 or comprising a heterologous DNA operably linked to a monocotyledonous promoter. But Stalker's lack of teaching of the claimed invention goes much further than this. Stalker generally teaches a new gene. To the extent plant transformation methods are taught by Stalker, they are merely recitations of known transformation methods that could be used to introduce a gene into a plant. So, for example, while Stalker teaches *Agrobacterium*-mediated transformation as one of many plant transformation protocols (col. 5, lines 38-55) and sets out a list of vectors, promoters and the like that were commonly used at the time (col. 5, line 55 through col. 6, line 51), and further lists a broad range of plants that could be transformed in accordance with one of the many listed methods, Stalker nowhere teaches or suggests, by statement or example, any specific instance of turfgrass transformation, by any method. The only plant transformation specifically taught by Stalker is a tobacco transformation (col. 21, line 33 through col. 22, line 66) using co-cultivation with *Agrobacterium*. Even in that example, tobacco leaf discs or cotyledon explants were taught as the co-cultivated plant material, not the method of using friable, regenerable callus tissue as claimed in the present application. For these reasons as well as the reasons acknowledged by the examiner, Stalker cannot be said to teach or suggest the invention as presently claimed.

The examiner asserts that Lee et al. provides one or more of the teachings missing from Stalker, namely, the use of a rice actin promoter, the production of transgenic turfgrass,

and *Agrobacterium*-mediated gene transfer. Applicants assert that a fair reading of Lee et al. reveals that this is not the case. Lee et al. teach a method for transforming monocotyledonous plants that, at its foundation, requires introduction of DNA directly into nodal tissue, and does not involve a co-cultivation method as claimed in the present invention. Indeed, Lee et al. teach away from the *Agrobacterium*-mediated co-cultivation for monocots such as turfgrass, by stating: [A]though transfer of foreign genes into plants by infection with *Agrobacterium tumefaciens* containing plasmid DNA is routine for many dicotyledons that readily form callus after wounding, the procedure is not routinely applicable in monocotyledons in which callus formation and transformation is more difficult" (col. 1, lines 40-45). Indeed, the method taught and claimed by Lee et al. is said to provide an alternative to the aforementioned method, by providing a means of transforming monocot plants that does not involve callus formation. Clearly, in this aspect, the methods taught by Lee et al. teach away from the method claimed in the instant application, which utilizes callus formation and use of callus in *Agrobacterium*-mediated transformation.

In addition, while Lee et al. teach generally that the transforming DNA may be "naked" DNA or part of an *Agrobacterium* vector and system, the latter embodiment is taught only in the context that "[A]ny method of introducing DNA into the cell of the node segment may be used in the invention . . ." (col. 6, lines 50-51). No *Agrobacterium*-mediated gene transfer is specifically taught by Lee et al.; therefore, it can hardly be said that Lee et al. teach *Agrobacterium*-mediated gene transfer in any meaningful way.

Furthermore, and notably, the specific teachings and examples set forth by Lee et al. relate only to the biolistic transformation method (e.g., col. 9, line 44 through col. 12, line 3). It is in this context only that the rice actin promoter is taught. Applicants assert that a teaching of the use of a monocot promoter in biolistic transformation cannot be said to be relevant to the method claimed in the present application, which is an entirely different method. The skilled artisan would not look to a biolistic transformation method for information pertinent to *Agrobacterium*-mediated co-culture methods.

Even assuming, *arguendo*, that Lee et al.'s use of an actin promoter in biolistic turfgrass transformation did provides some suggestion to use such a promoter in an *Agrobacterium* system, that suggestion would be merely be an invitation to try the promoter – there is no teaching that would impart any reasonable expectation that such a modification

would be successful in achieving *Agrobacterium*-mediated gene transfer into heretofore recalcitrant turfgrasses.

In sum, Stalker teaches only general examples of plant transformation and nowhere teaches turfgrass or monocot transformation by any method, and (2) Lee et al. teach a turfgrass transformation method that features direct gene transfer into nodal tissue and the preferred use of the biolistic technique, which is entirely different from the claimed method. Accordingly, there can be no motivation found in the cited references to combine the teachings of those references to arrive at the presently claimed invention. Teachings of many claimed elements are missing from the two references, and neither is of particular relevance to the problem solved by the claimed invention, which is to accomplish gene transfer into turfgrass by an *Agrobacterium*-mediated co-culture methodology. Therefore, the skilled artisan reviewing the cited references could not have been motivated in any way to combine their teachings or to make the many other modifications needed to arrive at the presently claimed invention.

The examiner states that Applicants' "admitted prior art," e.g., the pSB1 and pSB4 *vir* genes and the transforming DNAs set forth in the specification, taken with the two cited references, would render the claimed invention obvious. Applicants disagree. In view of the many teachings needed to arrive at the claimed invention that are so clearly missing from the cited references, it is difficult to imagine how the "admitted prior art" of pSB1 and pSB4 *vir* genes or transforming DNAs of interest could provide the teaching or motivation to combine those teachings to make the claimed invention.

Furthermore, and of particular significance, since the cited references do not provide any teaching or motivation to combine them to arrive at the invention as claimed, that motivation must have come from Applicants' disclosure, and not from the cited references themselves. Such use of Applicants' disclosure to glean the motivation to modify prior art teachings constitutes hindsight reasoning of a type that is impermissible to support an obviousness rejection. MPEP §2145.

For each of the reasons set forth above, the presently claimed invention clearly cannot be said to be obvious in view of the cited references. Applicants therefore request reconsideration and withdrawal of the rejections under 35 U.S.C. §103(a).

Conclusion:

Applicants believe this paper to be fully responsive to all outstanding issues. In view of the amendments presented herewith and the foregoing remarks, the claims are considered to be in condition for allowance and the same is earnestly sought in an early and favorable action. If further discussion would facilitate advancement of this application to allowance, the examiner is invited to contact the undersigned attorney at the telephone number provided below.

Respectfully submitted,

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